Original Research Article

EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF TABERNAEMONTANA DIVARICATA L. FLOWERS IN WISTAR ALBINO RATS

Sayyed Mateen[1], Amjad Ali[2], Allahbaksh Shaikh[3], Javed Akhatar Ansari[4], Imtiyaz Ansari[5*]

[1]Associate Professor, Dept. of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai -400705, India. Email: [1]mateen.sayyed@ocp.edu.in
[2]Associate Professor, Dept. of Pharmaceutical Chemistry, Oriental College of Pharmacy, Sanpada, Navi Mumbai -400705, India.
[3]Associate Professor and Principal, Dept. of Pharmacology, Delight College of Pharmacy, Bheema-koregaon.
[4]Professor , MESCO College of Pharmacy, Hyderabad
[5*]Assistant Professor and HOD, Dept. of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai -400705, India.

[*]Corresponding Author: Imtiyaz Ansari
[*]Assistant Professor and HOD, Dept. of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai -400705, India, E-mail: imtiyaz.ocp@gmail.com

Abstract:

Objective: To evaluate Anti-Arthritic activity of Ethanolic Extract of Tabernaemontana *divaricata L. Flowers* (EETF).

Method: Natural based products with therapeutic properties has become an important field of interest in many countries. Animals were injected with 0.1 ml of CFA (Complete Freund's Adjuvant) intradermally. Ethanolic extract of *Tabernaemontana divaricata L. Flowers* (*EETF*) treated at different concentration i.e. 100 mg/kg, 150 mg/kg, 200 mg/kg orally. The rats body weight, paw volume using digital plethysmometer, stair case climbing, motility, hematological parameter, radiography, histopathology was measured.

Result: The analysis of various arthritic assessment parameters used in this study revealed that Ethanolic Extract of *Tabernaemontana divaricata L. Flowers* (*EETF*) have considerable effect in preventing development of arthritis disease severity. Moreover, Ethanolic Extract of *Tabernaemontana divaricata L. Flowers* (*EETF*) revealed significant anti arthritis activity in both normal and CFA induced arthritis rats.

Conclusion: Ethanolic Extract of *Tabernaemontana divaricata L. Flowers* (*EETF*) shows pharmacological rationale for the traditional use of the plant against inflammatory conditions like rheumatoid arthritis.

Keywords: Complete Freund's Adjuvant(CFA), Body Weight, Paw Volume, Stair case, Motility.

1. INTRODUCTION

Animal model of inflammatory disease are used extensively in analysis of pathologic process of inflammatory arthritis and within pharmaceutical industry in testing of potential anti arthritic agents [1,2]. Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects primarily the joints manifesting as pain, stiffness, and synovitis (inflammation of the synovial membrane) leading in turn to particular destruction [3,4,5] . The risk of arthritis increases with increasing age. RA will occur at any age, however in men onset before age forty five years is uncommon [6] . The ultimate goal of RA treatment is to stop or atleast minimize joint damage, alleviate pain and maintain normal joint function [7,8] .

Trema orientalis plant is evergreen medium sized of family, *Ulmaceae or Cannabaceae*. Species in this family are significant in food industry as well as in pharmacologically as a traditional medicine in various ailments. It is valued for plup wood production and widley used as medicine in various parts of Africa. Common names: Indian Charcoal Tree, Indian Nettle, Oriental nettle, Pigeon wood. Vernacular names: Marathi- Ghol & kapshi, Telugu- Kakamushti, Hindi-Gio,Chikan,Gol,Gorklu, Sanskrit-Jivanti [9]. The young leaves are eaten as spinach by the Zulus in South Africa, who also use the roots and stem bark as traditional medicine. The fruit, leaves, bark, stem, twig and seeds are extensively used in traditional medicine [10].

2. MATERIAL AND METHOD:

Animals:

Male Wistar Albino Rats (100-150 g) were used for the study. The animals were obtained from Bombay Veterinary College Parel, Mumbai 400012. The use of these animals and the study protocols were approved by CPCSEA recognized institutional animal ethics committee (IAEC). Rats were kept at the animal house of Oriental College of Pharmacy, Sanpada, Navi Mumbai. In polypropylene cages, at 27± 3°C, maintain under Standard condition dark: light cycle. They were provided with commercial rat feed and water given ad libitum.

Animals and experimental design:

After 1 week of acclimatization the rats were randomized in different groups (n=6). 6 rats served as normal control (Group 1). 30 animals were immunized and injected with 0.1 ml of CFA on day 0, intradermally at the base of the tail and randomized into 5 different groups. Out of which 6 animals served as positive control (Group II), which did not receive any treatment and remaining animals were divided into Group III, IV, V and VI (6 animals each) which received treatment.

3. RESULTS AND DISCUSSION

Anti-Arthritic Study:

Body Weight: The body weight of animals for all groups was monitored from 0thday till 27thday at three days of interval i.e. 0,3,6,9,12,15,18,21,24,27 [11,12,13]. Results are reported in (Table 1) and (Fig. 1)

Percent change in body weight was calculated by formula:

$$\frac{\text{Body weight on day (X) - Body weight on day (0)}}{\text{Body weight on day (X)}} \times 100$$

Table 1: Results of % Changes in Body Weight

Days post immunization	% Change in Body Weight					
	Groups					
	Normal control	Disease control	Indomethacin (2.5mg/kg)	Low Dose (100 mg/kg)	Meidum Dose (150 mg/kg)	High Dose (200mg/kg)
0	0	0	0	0	0	0
3	8.27±2.06	5.92±1.0	3.66±0.46	2.77±0.32	5.35±0.53	4.87±1.25
6	13.84±1.37	10.27±0.88	9.26±0.82	6.80±0.24	8.70±0.77	8.57±1.45
9	18.17±1.50	13.91±0.84	14.34±0.98	15.66±0.47	13.44±1.23	13.26±1.90
12	22.93±0.85	8.20±1.10***	11.26±0.91	10.63±0.46	9.43±1.69	10.55±2.06
15	26.08±0.83	6.30±1.16***	8.04±0.69	4.06±1.26	7.19±1.71	7.21±2.39
18	28.78±0.83	4.34±1.14***	12.74±1.22#	13.31±1.11#	11.76±1.62	11.02±1.87
21	31.60±0.82	0.76±0.28****	17.03±1.47###	8.66±2.07	16.02±1.58##	14.08±1.71##
24	34.49±0.92	2.60±1.15****	20.74±1.28###	14.99±1.57#	19.57±1.26##	18.28±1.81##
27	38.50±0.70	6.57±1.31****	23.80±0.39###	17.84±0.96##	22.40±1.01##	22.58±1.66##

All measurements are expressed as Mean ± SEM with n=6 for each group. Values were considered significant when $p<0.05$ (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, p<0.05, **, ## p<0.01, ***, ### p<0.001, ****, ####p<0.0001.

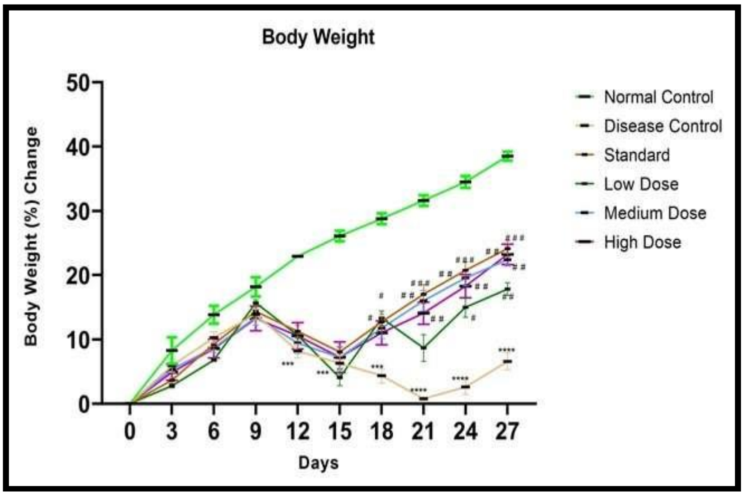


Fig. 1: % change in body weight

Paw Volume: The paw volumes were measured using Digital Plethysmometer (VJ instrument).Volumes of both right and left hind paws were taken on 0th,3rd,6th,9th,12th,15th,18th,21st,24th,27th day [14].Results are in the (Table 2,3) and (Fig.2,3). Percent change in paw volumes calculated by formula:

$$\frac{\text{Paw volume on day X (ml)} - \text{Paw volume on day 0(ml)}}{\text{Volume on day X (ml)}} \times 100$$

Table 2: Result of % Change in Right Hind Paw Volume

Days post immunization	% Change in Right Hind Paw Volume					
	Groups					
	Normal control	Disease control	Indomethacin (2.5mg/kg)	Low Dose (100 mg/kg)	Meidum Dose (150 mg/kg)	High Dose (200mg/kg)
12	10.09±1.11	15.44±0.66**	14.90±0.25	19.18±0.82	19.86±0.57	15.15±0.87
15	11.78±1.31	20.06±0.86**	19.76±0.23	22.03±1.26	22.30±0.64	18.64±0.90
18	12.95±1.34	23.55±1.21**	18.32±0.24#	24.39±1.27	24.27±1.51	21.70±1.36
21	32.98±1.38	27.52±1.27***	17.59±0.33##	26.35±1.82	21.35±0.99#	19.97±1.47#
24	14.66±1.37	30.81±1.10***	16.56±0.41###	28.42±1.41	18.53±0.41###	17.58±0.99###
27	15.32±1.36	28.88±1.14***	15.51±0.36###	26.90±1.39	16.54±0.40##	16.00±0.81##

All measurements are expressed as Mean ± SEM with n=6 for each group. Values were considered significant when p<0.05 (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, p<0.05, **, ## p<0.01, ***, ### p<0.001, ****, ####p<0.0001.

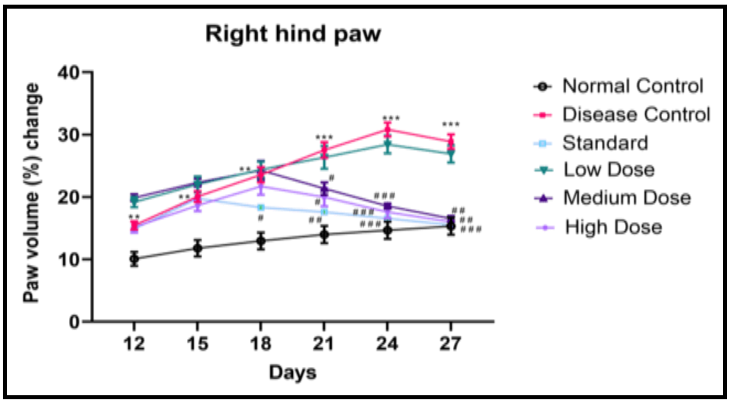


Fig. 2: % Change in Right Hind Paw Volume

Table 3 : Result of % Change in Left Hind Paw Volume

Days post immunization	**% Change in Left Hind Paw Volume**					
	Groups					
	Normal control	**Disease control**	**Indomethacin (2.5mg/kg)**	**Low Dose (100 mg/kg)**	**Meidum Dose (150 mg/kg)**	**High Dose (200mg/kg)**
12	10.87±2.08	23.38±0.43**	19.66±0.82#	16.92±1.88	19.12±0.84#	17.41±1.38#
15	11.87±2.05	38.31±2.54**	22.16±0.97##	33.24±2.21	24.12±1.18##	24.10±1.67#
18	13.22±1.86	53.04±2.05***	21.19±1.02####	48.05±1.08	40.36±3.66#	14.22±1.37#
21	15.09±1.84	57.97±1.59***	20.22±1.04####	55.91±1.15	38.59±3.11##	37.80±1.26###
24	16.89±1.78	63.83±2.19***	18.52±1.17####	54.65±1.34#	37.34±3.16###	35.61±1.19###
27	18.18±1.75	51.91±2.73***	17.35±1.43####	49.17±2.61	35.28±2.55##	32.28±1.45###

All measurements are expressed as Mean ± SEM with n=6 for each group. Values were considered significant when $p<0.05$ (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, $p<0.05$, **, ## $p<0.01$, ***, ### $p<0.001$, ****, ####$p<0.0001$.

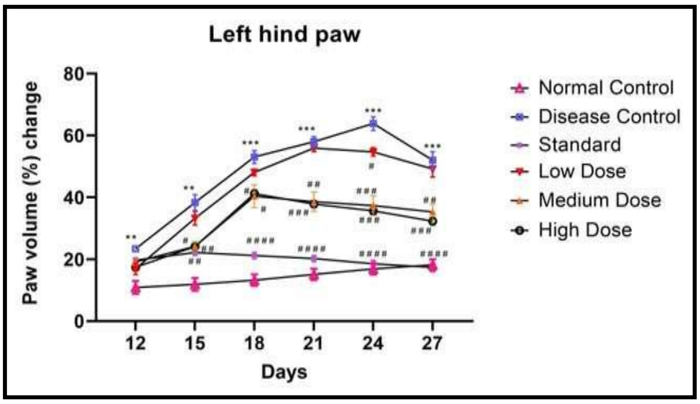


Fig. 3: % Change in Left Hind Paw Volume

Stair case climbing: Overnight fasted animals were trained for one week to climb a staircase with steps at height of 5, 10, and 15 cm having water at the second and food at the third step. Climbing ability of the rats groups was scored 0 if the rats did not climb; 1, if the rats climbed onto step 1; 2, if the rats climbed onto step 2 and 3, if the rat could climb all the three steps. All the groups were evaluated in this manner on 14th, 21st and 27th day of treatment period [15].Results in (Table 4) and (Fig. 4).

Table 4: Results of Stair case Climbing

Days post immunization	Stair case Climbing Score					
	Groups					
	Normal control	Disease control	Indomethacin (2.5mg/kg)	Low Dose (100 mg/kg)	Meidum Dose (150 mg/kg)	High Dose (200mg/kg)
14	3.00±0.00	0.67±0.21***	1.33±0.33	0.67±0.21	1.33±0.33	1.33±0.33
21	3.00±0.00	0.17±0.17****	3.00±0.00####	0.50±0.22	1.50±0.22##	2.83±0.17###
27	3.00±0.00	0.17±0.17****	3.00±0.00####	0.67±0.21	2.50±0.22##	3.00±0.00####

All measurements are expressed as Mean ± SEM with n=6 for each group. Values were considered significant when p<0.05 (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, p<0.05, **, ## p<0.01, ***, ### p<0.001, ****, ####p<0.0001.

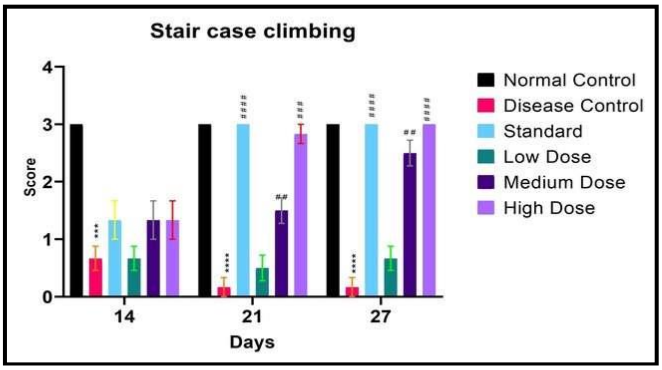


Fig. 4: Stair case Climbing

Motility : The rats were observed for a period of minutes and given scores. Scored 2, if the rat avoided touching the inflamed joint to the floor while walking; 1, if the rat walked with little difficulty, but the toe touched the floor; 0, if the rat walked easily. All the groups were evaluated in this manner on 14th, 21st and 27th day of treatment period [16].Results in (Table 5) and (Fig. 5).

Table 5: Results of Motility

Days post immunization	Motility					
	Groups					
	Normal control	Disease control	Indomethacin (2.5mg/kg)	Low Dose (100 mg/kg)	Meidum Dose (150 mg/kg)	High Dose (200mg/kg)
14	0.00±0.00	2.00±0.00****	2.00±0.00	0.67±0.21	2.00±0.00	2.00±0.00
21	0.00±0.00	2.00±0.00****	0.33±0.21##	2.00±0.00	1.17±0.17#	0.50±0.22##
27	0.00±0.00	2.00±0.00****	0.00±0.00####	2.00±0.00	1.17±0.17#	1.17±0.17###

All measurements are expressed as Mean ± SEM with n=6 for each group. Values were considered significant when p<0.05 (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, p<0.05, **, ## p<0.01, ***, ### p<0.001, ****, ####p<0.0001.

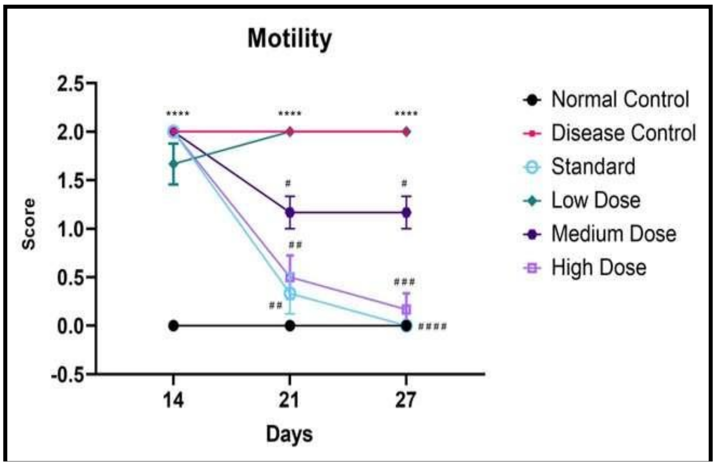


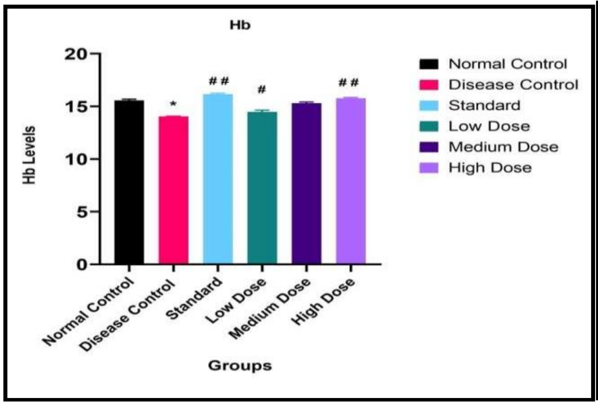
Fig. 5: Motility

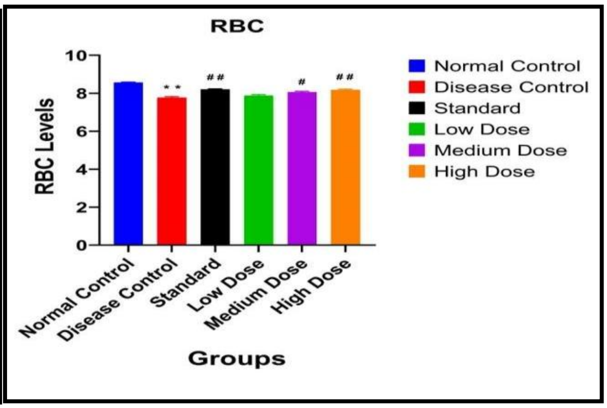
Hematology: On 27thday one day before sacrifice, blood samples were withdrawn from retro orbital vein puncture under mild anaesthesia and kept under suitable blood collection tube (BD Vacutainer®) and submitted to Dr.D.P.Chaudhari,Parel. Hematological parameter includes Hemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC) [17,18,19].Results in (Table 6) and (Fig.6).

Table 6: Results of Hematology

Hematology	Groups					
	Normal control	**Disease control**	**Indomethacin (2.5mg/kg)**	**Low Dose (100 mg/kg)**	**Meidum Dose (150 mg/kg)**	**High Dose (200mg/kg)**
Hb	15.57 ± 0.12	$14.03\pm0.03^{*}$	$16.17\pm0.09^{\#\#}$	14.47 ± 0.18	$15.30\pm0.12^{\#}$	$15.77\pm0.09^{\#\#}$
RBC	8.57 ± 0.02	$7.78\pm0.05^{**}$	$8.21\pm0.03^{\#\#}$	7.89 ± 0.05	$8.07\pm0.04^{\#}$	$8.18\pm0.04^{\#\#}$
WBC	17.43 ± 0.15	$20.07\pm0.12^{**}$	$7.60\pm0.23^{\#\#\#\#}$	$18.00\pm0.06^{\#}$	$17.23\pm0.19^{\#}$	$16.50\pm0.12^{\#\#}$

Values are expressed as Mean ± SEM with n=3 for each group. Values were considered significant when $p<0.05$ (95 % confidence interval). Two way ANOVA followed by Tukey's test and multiple comparison was made between different groups. * significantly different when compared with normal control group, # significantly different when compared with disease control group. *, #, $p<0.05$, **, ## $p<0.01$, ***, ### $p<0.001$, ****, ####$p<0.0001$.





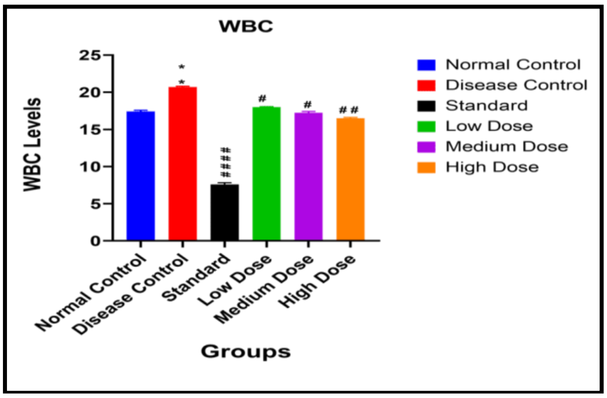


Fig. 6 Hb,RBC,WBC levels

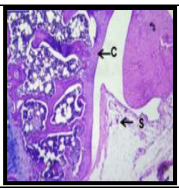
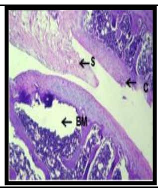
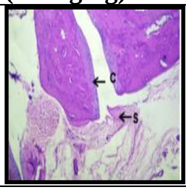
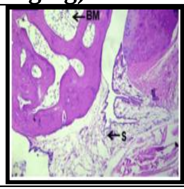
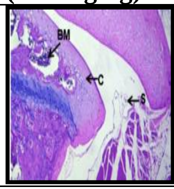
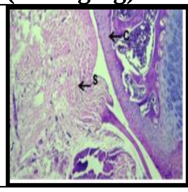
Radiography: Radiographs of the ankle joints were taken on 27th day, a day before euthanasia. Plain radiographs of the ankle joints were taken at the Petit Hospital for Animals,Parel . Rats were anesthetized before taking radiographs using light ether anesthesia [20].Results in (Table 7).

Table 7 : Results of Radiography

Normal control	**Disease control**	**Indomethacin (2.5mg/kg)**	**Low Dose (100 mg/kg)**	**Meidum Dose (150 mg/kg)**	**High Dose (200mg/kg)**
					
Radiographic analysis of the normal control rats showed normal architecture of the ankle joints. No bony erosions, ankylosis, soft tissue swelling and new bone formation were observed.	Radiographic analysis of the disease control rat immunized with CFA shown bony erosions to a higher degree when compared to normal control group. Soft tissue swelling were observed. Ankylosis of the ankle joints were observed. New bone formation was also observed to a minimal degree.	Radiographic analysis of rats treated with Indomethacin (2.5mg/kg/day) shown minimal soft tissue swelling when compared with normal control group. New bone formation, bony erosions and joint alignment changes were not observed.	Radiographic analysis of the rats with *EETF* (100mg/kg/day) revealed severe degree of soft tissue swelling when compared with normal control group. Ankylosis and joint space narrowing was minimal when compared to disease control group.	Radiographic analysis of rats treated *EETF* (150mg/kg/day) shown moderate soft tissue swelling when compared to disease control rats. Changes in bone architecture,joint space narrowing, erosions were observed to a lesser degree when compared to disease control group.	Radiographic analysis of the group treated with *EETF* (200mg/kg/day) shown a minimal degree of soft tissue swelling when compared with disease control rats. Bone architecture was retained with *EETF* (200 mg/kg/day) treated rats.

Histopathology: Animals were sacrificed on 28^{th} day, Ankle joints isolated for histopathological evaluation were stored in 10% neutral buffered formalin solution (10ml of formaldehyde was made up to 100ml with phosphate buffer solution, pH 7.4) submitted to Astha Laboratory,nerul. Tissue were prepared and stained with haematoxylin and eosin stain. Slides was subjected to light microscopic analysis for evaluation of Infilteration of inflammatory cells in synovium, cartilage damage, Bone marrow depletion [21,22,23].Results in (Table 8).

Table 8 : Results of Histopathology

Normal control	Disease control	Indomethacin (2.5mg/kg)	Low Dose (100 mg/kg)	Meidum Dose (150 mg/kg)	High Dose (200mg/kg)
					
Normal architecture of the ankle was observed. Histopathological changes in ankle joint of Adjuvant induced arthritis such as synovial cell derrangement and hyperplasia,bone and cartilage degradation, inflammatory cell infiltration were absent.	When compared to Normal Control Group, it was observed that Moderate Synovial hyperplasia and Cartilage damage was seen in ankle joints of rats injected with complete Freund's adjuvant. Mild bone marrow was also observed.	When compared with disease control group, it was observed that the articular cartilage destruction and Infiltration of Inflammatory cells of Synovium was restored to normal. It was also observed that Bone marrow was mild in comparison to disease control rats. Improvement towards normal architecture of the ankle joint was observed.	Infiltration of Inflammatory cells of Synovium was mild observation seen. Bone marrow destruction was observed to moderate degree,when compared to Indomethacin treated group.	Bone marrow and cartilage destruction was reduced to a mild extent when compared with disease control group. Minimal Infiltration of Inflammatory cells of Synovium were evident from histopathological observations.	Cartilage and Infiltration of Inflammatory cells of Synovium were minimal when compared with disease control group. The Bone marrow was restored to normal in comparison with disease control.

4. CONCLUSION

The findings of the current study indicate that Ethanolic Extract of *Tabernaemontana divaricata L. Flowers EETF*(200 mg/kg) possess anti-arthritic activity.

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Conflict of Interest

The creators announce that there are no irreconcilable circumstances with respect to the production of this paper.

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